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Calpain-1 and calpain-2: the yin and yang of synaptic plasticity and neurodegeneration

Michel Baudry and Xiaoning Bi

Western University of Health Sciences, Pomona, CA 91766

Abstract

Many signaling pathways participate in both synaptic plasticity and neuronal degeneration. While calpains participate in these phenomena, very few studies have evaluated the respective roles of the two major calpain isoforms in the brain, calpain-1 and calpain-2. We review recent studies indicating that calpain-1 and calpain-2 exhibit opposite functions in both synaptic plasticity and neurodegeneration. Calpain-1 activation is required for the induction of long-term potentiation (LTP) and is generally neuroprotective, while calpain-2 activation limits the extent of potentiation and is neurodegenerative. This duality of functions is related to their associations with different PDZ binding proteins, resulting in differential subcellular localization, and offers new therapeutic opportunities for a number of indications in which these proteases have previously been implicated.

Keywords

calpain; plasticity; learning; neuroprotection; neurodegeneration; protein synthesis

Common pathways in synaptic plasticity and neurodegeneration

All cells in multicellular organisms have to perform a number of basic functions: to reproduce, grow, migrate, respond and adapt to external stimuli, and to survive (or die). Not surprisingly, these functions require a large number of house-keeping genes and regulatory systems. Neurons share all these functions, with the exception of reproduction, and face the additional challenge that they need to be better able to respond and adapt to a wide array of internal and external signals, and to survive despite multiple insults and the deleterious effects of the aging process [1]. Furthermore, neurons had to evolve adaptations of these mechanisms in specialized cell compartments needed for neuronal communication, the synapses. Thus, neuronal and synaptic plasticity, the ability of neurons or synapses to adapt to changes in the environment, and neuronal survival or death, i.e., neurodegeneration, are processes that have evolved in parallel using the cellular machinery involved in basic cellular

Send proofs and correspondence to: Michel Baudry, GCBS, Western University of Health Sciences, 309 E. 2nd St, Pomona, CA 91766, mbaudry@westernu.edu, Tel: 909-469-8271.

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physiological functions, including gene regulation, cytoskeletal adaptation, and neuronal survival/apoptosis [2]. Numerous studies indicate that synaptic plasticity and neurodegeneration are indeed two sides of the same coin, and that many signaling pathways are involved in both synaptic plasticity and learning and memory and in synaptic dysfunction and neurodegeneration [3–5]. These pathways, including histone acetylation and deacetylation [6–8], Wnt- β -catenin [9–12] and mTOR signaling [13, 14], are implicated in gene regulation, local protein translation and degradation, as well as in cytoskeletal remodeling.

This review will focus on a signaling pathway, the calpain system, which was previously shown to be critical for cell motility and cell division. It appears that this pathway has been adapted through evolution to play a major role in both synaptic plasticity and neuronal survival/neurodegeneration [15]. Calpain is an evolutionary old family of soluble, neutral, calcium-dependent proteases (Box 1), which have the unique property of using protein cleavage to modify the activity/function of their substrate proteins. As such, they constitute a post-translational regulatory mechanism, which is irreversible and long-lasting, since it lasts for the life of the protein. Specifically, we will focus on the remarkable findings over recent years that the two major calpain isoforms in the brain, calpain-1 and calpain-2 (Box 1), play opposite roles in synaptic plasticity and neuronal survival/neurodegeneration.

Box 1

General features of calpains

I. Vertebrate evolution of the calpain family

Calpains are generally divided into typical (or classical) and atypical (or non-classical) calpains, where the typical calpains exhibit a penta-EF-hand type of calcium binding domain, which is lacking in atypical (or non-classical) calpains. Most initial studies have focused on calpain-1 and calpain-2, which are also called the conventional calpains [85, 86].

II. Domain structure of typical calpains

To the 16 calpain genes has been added *CAPNS1*, a gene coding for a small subunit (aka calpain-4) common to calpain-1 & 2. This gene has a glycine-rich domain (GR) and a PEF domain (referred to PEF (S) in the small subunit. Typical calpains have an alpha-helix in the N-terminal domain, followed by two protease core domains (PC1 and PC2, also known as the CysPC domain). Previously referred to as domain III is now called C2-domain-like domain (C2L), while the previously called domain IV is a PEF domain in typical calpains PEF (L) (in the long subunits). Table illustrates the strongly conserved 4 amino acids in the C-terminal domains of calpain-1 and calpain-2, which constitute a Type II or Type I PDZ-binding domains, across several vertebrate species [87, 88]. Note that calpain-1 and calpain-2 were previously referred to as μ -calpain and m-calpain, in view of their calcium requirements for in vitro activation.

III. Domain structure of atypical calpains

As mentioned above, atypical calpains lack either the C2L or the PEF domains. In addition, calpain-7 and calpain-10 have a Microtubule interacting and trafficking motif (MIT). Calpain-15 has a Zn-finger motif domain (Zn) and a Small Optic Lobe (SOL) homologous domain (SOH). Calpain-16 is also called half-calpain, as it exhibits only one PC domain.

Opposite roles of calpain-1 and calpain-2 in synaptic plasticity and learning and memory

Demonstrating a role for calpain in LTP

Calpain's participation in long-term potentiation (LTP) and learning and memory was first proposed in 1984 [16]. Although the existence in the brain of calpain-1 and calpain-2 was established at that time, it was not known which isoform was involved in LTP and which target protein(s) was(were) critical for producing the functional changes underlying LTP (except for the potential role of spectrin degradation and its involvement in structural modifications [17]. Experiments using down-regulation of calpain-1 by treating cultured

hippocampal slices with siRNA suggested calpain-1 was involved in LTP [18], although the use of hippocampal slices prepared from calpain-1 knock-out mice did not initially confirm this result [19]. On the other hand, down-regulation of the endogenous calpain inhibitor, calpastatin, decreased the threshold for LTP induction [20].

Over the last 10 years, several findings have indicated that calpain-1 and calpain-2, by cleaving several proteins, participate in the regulation of dendritic structure and local protein synthesis. The first hint that calpain was linked to the regulation of local protein translation was provided by a report that calpain cleaved dicer and released dicer and eIF2c from postsynaptic densities, thereby facilitating the processing of miRNA and regulating protein translation [21]. It was later reported that calpain cleaved and inactivated the suprachiasmatic nucleus circadian oscillatory protein (SCOP, aka PH domain and Leucine rich repeat Protein Phosphatase 1 (PHLPP1 β)) [22], a negative regulator of the extracellular signal regulated kinase (ERK), a kinase with numerous links to LTP [23], thus linking calpain activation to activation of the ERK pathway. Another pathway critical for synaptic plasticity involves the protein kinase Cdk5 [24, 25]. In particular, Cdk5 binds to calpain and NR2B, facilitating calpain-mediated cleavage of NR2B [26]. In addition, calpain cleaves the Cdk5 activator p35 to produce a more potent activator, p25 and this event also participates in synaptic plasticity [27]. Calpain was also shown to cleave β -catenin, generating an active fragment, which regulates gene transcription, thereby providing a mechanism by which NMDA receptor stimulation, which has been repeatedly linked to calpain activation [28, 29], could modify gene expression [30]. Several scaffolding proteins were found to be calpain substrates, including PSD95 [31], GRIP [32], SAP97 [33] and ARMS (ankyrin repeat-rich membrane spanning protein) or Kidins220 (kinase D-interacting substrate of 220 kDa) [34]. By degrading the translational repressor poly(A)-binding protein (PABP)-interacting protein 2A (PAIP2A), an inhibitor of PABP, calpain could also relieve translational inhibition of proteins involved in synaptic plasticity and learning and memory [35]. More recently, the role of calpain in LTP received strong support from results of experiments using hippocampal slices from mice with a conditional down-regulation of calpain-4, the small subunit required by both calpain-1 and calpain-2 for functional activity, since the lack of calpain activity resulted in impairment in LTP induction [36].

Parceling out the role of calpain-1 and calpain-2 in LTP

Two major findings changed the view of the roles of calpain-1 and calpain-2 in LTP. First, BDNF, a neurotrophic factor critically involved in synaptic plasticity and in LTP [37], was found to activate calpain-2 through ERK-mediated phosphorylation at Serine 50 [38]. In addition, BDNF-induced stimulation of local protein synthesis, a critical process in LTP formation [39], was mediated by calpain-2 activation of mTOR [40]. Detailed analysis of this mechanism indicated that calpain-2, but not calpain-1, cleaved the phosphatase PTEN, a negative mTOR regulator, thus suggesting that calpain-2 activation following LTP induction could lead to increased local protein synthesis and participate in LTP consolidation.

The other finding illuminating the roles of calpains in LTP came from experiments using a broad spectrum, cell-permeable calpain inhibitor, calpain inhibitor III, applied either before or after delivering theta burst stimulation (TBS) in acute hippocampal slices. These results

were quite remarkable, as applying calpain inhibitor III before TBS inhibited LTP, as previously reported [41, 42], while applying it immediately after TBS enhanced the magnitude of LTP; treatment with calpain inhibitor III one h after TBS had no effect [43]. Understanding these surprising results required combining the effects of calpain-1 on PHLPP1 β degradation/inactivation and calpain-2 on mTOR-mediated protein synthesis. Specifically, rapid calpain activation following TBS resulted in PHLPP1 β degradation and ERK activation. However, the rapid PHLPP1 β degradation was followed by its rapid de novo synthesis, thereby limiting the duration of ERK activation [43]. This rapid synthesis required calpain-2-mediated PTEN degradation and mTOR activation and stimulation of protein synthesis. In support of this model, a selective calpain-2 inhibitor, the dipeptide ketoamide, Z-Leu-Abu-CONH-CH₂-C₆H₃(3,5-(OMe)₂), [44] had no effect on LTP induction when applied before TBS, but produced the same increase in LTP magnitude starting about 10 min after TBS [43].

All these results led to a new model for LTP induction and consolidation following TBS (Fig. 1). In this model, calpain-1 is rapidly activated following NMDA receptor stimulation triggering the signaling cascade initiating the changes in synaptic structure leading to LTP. In contrast, calpain-2 activation acts as a molecular brake, which limits the extent of potentiation following a single TBS episode, and provides a plausible explanation for recently discovered timing rules for LTP [45]. Thus, calpain-2 activation serves to restrict single trial encoding of new information to a subset of synapses and imposes a delay before the initial representation can be expanded. This mechanism could be directly related to the well-known distributed practice effect, a fundamental aspect of learning that has received little attention from neuroscientists. Specifically, training trials spaced apart by sizable delays produce much stronger memory than does a single, massed learning session [46].

The widespread role of calpain in synaptic plasticity

In our new model of the calpain cascades (Fig. 1), we also incorporated other signaling pathways which have been demonstrated to participate in LTP induction and consolidation (Table I). We recently reviewed the mechanisms linking TBS to cytoskeletal regulation involved in LTP consolidation; in particular the pathways connecting TBS to actin polymerization in dendritic spines [47]. Two actin-signaling pathways involve the Rho family of small GTPases, RhoA, Rac and Cdc42, which are ubiquitously implicated in the mechanisms of actin filament assembly, disassembly, or stabilization in most cells [48], and have been shown to be critically involved in LTP consolidation [49, 50]. Calpain-1 and calpain-2 also have opposite effects on RhoA, thereby providing another mechanism linking calpain activation to regulation of actin filaments and the dendritic spine cytoskeleton [51]. Furthermore, it also has been recently shown that p70S6K, a kinase downstream of mTORC1, can phosphorylate p21-activated kinase (PAK) [52], which would provide another link between the calpain cascade and regulation of actin polymerization. Finally, calpain-2 has also been shown to degrade the Striatal-Enriched protein phosphatase (STEP) [53, 54], an enzyme involved in AMPA receptor endocytosis [55]. Thus, calpain cascades are linked to many of the events that have been proposed to participate in synaptic plasticity, including cytoskeletal regulation, AMPA receptor trafficking, actin polymerization and regulation of local protein synthesis.

Opposite roles of calpain-1 and calpain-2 in neuroprotection/ neurodegeneration

While there is an abundant literature supporting the role of calpain in neurodegeneration, there is a paucity of information regarding the respective roles of calpain-1 and calpain-2 in this process, as well as the nature of the calpain targets that participate in neurodegeneration. Furthermore, while overactivation of calpain has been implicated in a wide range of pathological states, including stroke, epilepsy, traumatic nerve injury, neurodegenerative disorders, and aging [56–58], a number of studies have reported opposite findings, indicating that calpain activation could also provide neuroprotection under certain conditions [59–61].

Involvement of calpain in tau hyperphosphorylation

A number of potential calpain targets have been proposed to play critical roles in certain forms of neurodegeneration (see Table 1). Like in the case of synaptic plasticity, a lot of emphasis has been placed on the relationship between calpain and Cdk5 [24, 62, 63], as Cdk5, which is normally activated by its specific activator p35, becomes hyperactivated under pathological conditions due to calpain-mediated cleavage of p35 to p25 [64–66]. Whether Cdk5/p25 is responsible for tau hyperphosphorylation under pathological conditions, including in Alzheimer's disease (AD) or frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), is still controversial; further studies are needed to provide a convincing link between calpain activation, Cdk5 hyperactivation and tau hyperphosphorylation [62]. Another link between calpain, tau hyperphosphorylation and AD is provided by calpain-mediated truncation of GSK-3 β [67–69]. Calpain truncates GSK-3 β in its C-terminal domain, resulting in increased kinase activity; in human AD brain, GSK-3 β truncation is correlated with tau hyperphosphorylation, tangle score and Braak stage [69]. However, increasing Ser9 phosphorylation by inhibiting certain phosphatases, such as phosphatase 1/2A prevented calpain-mediated cleavage of GSK-3 β [68], indicating that the relationship between calpain, GSK-3 β and tau hyperphosphorylation is extremely complex. Furthermore, another protein kinase, dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A), has been shown to exhibit enhanced activity towards tau phosphorylation as a result of calpain-mediated cleavage [70]. Finally, down-regulation of calpastatin enhanced tau neurotoxicity, an effect that is abolished by overexpression of calpastatin [71]. Calpain has also been proposed to participate in amyloid β peptide (A β)-mediated neurotoxicity through its truncation and inactivation of one of the sodium calcium exchangers, NCX3 [72]. In AD brain, levels of A β 1-42 were correlated to those of calpain-mediated truncated NCX3, although an opposite mechanism, enhanced function of NCX3 resulting from calpain-mediated truncation was also reported [61]. It is important to note that most of these studies did not attempt to determine the respective roles of calpain-1 and calpain-2 in the processes under evaluation.

Differential effects of calpain on neuronal survival are mediated via different NMDAR signaling

The effects of NMDA receptor stimulation vary with receptor localization; activation of synaptic NMDARs provides neuroprotection, while activation of extrasynaptic NMDARs is

linked to pro-death pathways [73]. PHLPP1 exhibits two splice variants, PHLPP1 α and PHLPP1 β , which share amino acid sequence similarity but have different sizes (140 kDa and 190 kDa, respectively). PHLPP1 α dephosphorylates Akt in neurons [74] and its down-regulation is related to cell survival in CNS [75–77]. PHLPP1 β , inhibits ERK by binding and trapping its activator Ras in the inactive form [78] and as discussed above is degraded by calpain in hippocampus; its degradation contributes to LTP and learning and memory. Relevant to its role in neuroprotection/neurodegeneration, synaptic NMDAR-induced activation of calpain-1 degraded both PHLPP1 α and PHLPP1 β , leading to activation of the Akt and ERK pathways and neuroprotection (Fig. 2). Calpain cleavage of PHLPP1 α and β was necessary and sufficient for synaptic NMDAR-induced activation of the Akt and ERK pathways since calpain inhibition blocked, while PHLPP1 knockdown mimicked, the effects of synaptic NMDAR activation on Akt and ERK pathways [49]. PHLPP1 suppresses Akt and ERK pathways under basal conditions; following synaptic NMDAR activation, calpain cleaves PHLPP1 α and β , thus releasing the inhibition of these two major pro-survival signaling cascades in neurons. Consistent with these results, PHLPP1 knockout mice are more resistant to ischemic brain injury [77]. Thus, PHLPP1 should be considered a novel potential target for the treatment of neurodegenerative diseases.

On the other hand, extrasynaptic NMDARs specifically activate calpain-2, which degrades STEP, ultimately resulting in neurotoxicity [53] (Fig. 2). Synaptic NMDAR activation did not result in degradation of PTEN, suggesting that synaptic NMDAR activation does not activate calpain-2. The use of calpain-1 and calpain-2 selective inhibitors confirmed this hypothesis: a calpain-2 selective inhibitor did not affect synaptic NMDAR-dependent PHLPP1 cleavage and neuroprotection but blocked extrasynaptic NMDAR-dependent STEP cleavage and neurotoxicity. In contrast, a calpain-1 selective inhibitor blocked synaptic NMDAR-mediated effects but not extrasynaptic NMDAR-mediated neurotoxicity. Similarly, calpain-1 knockout blocked NMDA-induced degradation of PHLPP1 but not STEP, inhibited ERK activation and exacerbated neurotoxicity in neonatal hippocampal slices. On the other hand, a calpain-2 selective inhibitor blocked NMDA-induced degradation of STEP and suppressed neurotoxicity in slices prepared from both wild-type and calpain-1 knock-out mice [49]. Thus, calpain-1 is preferentially activated by synaptic NMDAR stimulation, whereas calpain-2 is preferentially activated by extrasynaptic NMDAR stimulation [49]. It is not surprising from the above that calpain-1 is localized in synaptic compartments [79], where it could regulate synaptic function through its action on synaptic elements such as cytoskeletal and scaffolding proteins as well as glutamate receptors [56]. Little is known regarding the ultrastructural localization of calpain-2 in neurons.

Co-immunoprecipitation experiments indicated that NR2A-containing NMDARs, PSD95, calpain-1 and PHLPP1 form a complex in neurons [54]. Furthermore, synaptic NMDAR activity recruited calpain-1 to this NMDAR multi-protein complex. In contrast, calpain-2 was not associated with this complex under basal conditions or recruited by activity, consistent with the absence of calpain-2 activation following synaptic NMDAR activation. Besides the extrasynaptic NMDAR-STEP pathway, it has been reported that SAP102 mediates the movement of NR2B-containing NMDARs from synaptic to extrasynaptic membranes [80], where calpain has been shown to cleave NR2B and disrupt its interaction with SAP102 under neurotoxic conditions [81]. As indicated in Box 1, calpain-1 and

calpain-2 exhibit different types of PDZ binding motifs, and it is therefore likely that these different binding sites are involved in the different scaffolding of calpain-1 and calpain-2 to distinct protein clusters.

These findings challenge the prevalent theory that the duration of calpain activation determines whether calpain plays physiological or pathological roles. Instead, these results support the notion that calpain function is determined by local scaffolding with upstream receptors and downstream substrates. In particular, work indicating that calpain-1 and calpain-2 are preferentially coupled to synaptic and extra-synaptic NMDARs, respectively, underlies the importance of considering specific calpain isoforms as potential clinical targets for the treatment of NMDAR-related neurodegenerative diseases.

Concluding Remarks and Future Perspectives

Although calpain has been implicated in synaptic plasticity and neurodegeneration for many years, it is only recently that it has been appreciated that calpain-1 and calpain-2 play opposite functions in both synaptic plasticity/learning and memory and neuroprotection/neurodegeneration. Recent research has demonstrated that calpain-1 activation is necessary for certain forms of synaptic plasticity and learning and memory, while calpain-2 activation during a brief consolidation period limits the extent of plasticity/learning. Similarly, calpain-1 is neuroprotective, while calpain-2 is neurodegenerative. The signaling pathways triggered by both isoforms overlap pathways previously identified as critical for each process (synaptic plasticity or neuroprotection/neurodegeneration). In particular, calpain-2 activation, through the selective degradation of PTEN, is linked to the regulation of mTOR-mediated local protein synthesis. This unique function of calpain-2 underscores the complex relationships between protein synthesis and protein degradation, which has been shown to be critical in numerous neurological and neuropsychiatric disorders [13, 82]. In agreement with the role of mTOR-mediated local protein synthesis in synaptic plasticity, rapamycin treatment has been shown to be beneficial in several animal models of learning impairments [83, 84].

Finally, the identification of the yin and yang functions of calpain-1&2 provides new potential therapeutic treatments for a variety of disorders associated with learning impairment and/or neurodegeneration. Thus, a selective calpain-2 inhibitor, by enhancing the extent of long-term potentiation, could enhance learning in disorders associated with learning impairment. Likewise, by preventing STEP truncation and activation of p38, the same inhibitor could limit neurodegeneration. A calpain-1 activator would mimic the effects of a selective calpain-2 inhibitor and also facilitate learning and stimulate neuroprotection.

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Trends

- Several signaling pathways are involved in both synaptic plasticity and neurodegeneration.
- Calpain-1&2 have opposite functions in synaptic plasticity and neurodegeneration, due to their associations with different downstream signaling cascades.
- Calpain-2 couples local protein synthesis and degradation, which play critical roles in both synaptic plasticity and neurodegeneration.
- These mechanisms offer new targets for therapeutic approaches to diseases associated with learning impairment and neurodegeneration.

Figure 1. Opposite functions of calpain-1&2 in synaptic plasticity

Calpain-1 is rapidly stimulated by the calcium influx generated by NMDA receptor activation, resulting in spectrin and PHLPP1 β (SCOP) degradation. Rapid calpain-1 activation triggers a number of different signaling pathways, including cytoskeleton reorganization due to spectrin and RhoA truncation, and the ERK pathway through PHLPP1 β degradation. In the following minutes, calpain-2 is in turn phosphorylated and stimulated through BDNF-mediated ERK activation; this leads to PTEN degradation, mTOR activation and stimulation of the synthesis of a number of proteins with mRNAs located in dendrites, including PHLPP1 β and RhoA. Restoration of normal PHLPP1 β levels inhibits ERK, thereby terminating the induction process for LTP.

Figure 2. Opposite functions of calpain-1&2 in neurodegeneration

Stimulation of synaptic NMDAR activates calpain-1, which cleaves PHLPP1, resulting in phosphorylation/activation of Akt and ERK, two pro-survival pathways. Stimulation of extrasynaptic NMDAR activates calpain-2, which cleaves and inactivates STEP, resulting in p38 stimulation and neurodegeneration. Calpains also cleave and activates GSK3 β , which phosphorylates tau and results in tauopathy. In addition, calpains cleave p35 into the more active p25 activator of cdk5, which also phosphorylates tau. It is not currently known which calpain is involved in GSK3 β and p35 truncation.

Table 1
Substrates of calpain-1 and calpain-2 and their involvement in synaptic plasticity and neurodegeneration.

Calpain substrates	Calpain-1	Calpain-2	Synaptic plasticity	Neurodegeneration	References
Dyrk1A				Tau phosphorylation	[70]
GSK3β				Increases Tau phosphorylation	[67–69]
NCX3				Changes Aβ1–42 levels	[72] [61]
p35-p25 conversion (Cdk5 regulator)			Associated with LTP induction	Increases Tau phosphorylation	[27] [64–66]
PTEN			Limits LTP		[40] [43]
RhoA			Required for LTP		[51]
PHLPP1			Required for LTP and learning	Induces neuroprotection through Akt and ERK activation	[22] [54]
Spectrin			Remodels neuronal structure	Disrupts neuronal structure	[17]
STEP			AMPA endocytosis	Triggers neurodegeneration	[53, 54] [55]

A number of proteins are cleaved by calpain-1 and calpain-2. With the exception of PTEN, it appears that they are cleaved by both calpain-1 and calpain-2 (at least in brain homogenates). Cleavage of these proteins has been associated with either synaptic plasticity/LTP as well as neurodegeneration. Further studies are needed to specify the roles of calpain-1 and calpain-2 mediated cleavage in these 2 processes.